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Mutation analysis and disease features at presentation in a multi-center cohort of children with monogenic cholestasis

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Conflicts of Interest (COIs)

RJT consults for: Albireo Pharma, Alnylam, EVOX Therapeutics, GenerationBio, Horizon Pharma, Mirum Pharma, Qing Bile Therapeutics, Retrophin, and Sana Biotechnology; he has share options in GenerationBio and Qing Bile Therapeutics. SPH reports participation in the Mirum maralixibat PFIC trials – research grants only paid to the institution. MSC reports participation in the Bolder Surgical, Inc. (Louisville, CO) Physician Advisory Board. KML declares consulting relationships and research grants from Mirum Pharmaceuticals and Albireo Pharma. BMK reports she is a consultant and has unrestricted educational grants from Albireo and Mirum. For all remaining coauthors, no COIs are declared.

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Abstract

Objectives: To advance our understanding of monogenic forms of intrahepatic cholestasis.

Methods: Analyses included participants with pathogenic biallelic mutations in *ABCB11* (bile salt export pump; BSEP) or *ATP8B1* (familial intrahepatic cholestasis; FIC1), or those with monoallelic or biallelic mutations in *ABCB4* (multidrug resistance; MDR3), prospectively enrolled in the Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis (LOGIC; NCT00571272) between 11/2007–12/2013. Summary statistics were calculated to describe baseline demographics, history, anthropometrics, laboratory values, and mutation data.

Results: Ninety-eight participants with FIC1 (n=26), BSEP (n=53, including 8 with biallelic truncating mutations [severe] and 10 with p.E297G or p.D482G [mild]), or MDR3 (n=19, including 4 monoallelic) deficiency were analyzed. Thirty-five had surgical interruption of the enterohepatic circulation (sEHC), including 10 who underwent liver transplant (LT) after sEHC. Onset of symptoms occurred by age 2 years in most with FIC1 and BSEP deficiency, but was later and more variable for MDR3. Pruritus was nearly universal in FIC1 and BSEP deficiency. In participants with native liver, failure to thrive was common in FIC1 deficiency, high ALT was common in BSEP deficiency, and thrombocytopenia was common in MDR3 deficiency. sEHC was successful after more than 1 year in 7 of 19 participants with FIC1 and BSEP deficiency. History of LT was most common in BSEP deficiency. Of 102 mutations identified, 43 were not previously reported.

Conclusions: In this cohort, BSEP deficiency appears to be correlated with a more severe disease course. Genotype-phenotype correlations in these diseases are not straightforward and will require study of larger cohorts.

Keywords

ATP8B1 ; *ABCB11* ; *ABCB4* ; liver transplant; cholestasis

INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) includes intrahepatic cholestatic monogenic disorders of multiple etiologies, now named based upon the dysfunctional protein. The first described, FIC1 deficiency (FIC1 [*ATP8B1*] disease, PFIC1), bile salt export pump (BSEP) deficiency (BSEP [*ABCB11*] deficiency, PFIC2), and multidrug resistance (MDR3) deficiency (MDR3 [*ABCB4*] disease, PFIC3), are the longest-studied and most common known forms of PFIC[1–3].

FIC1 and BSEP deficiency often present in the neonatal period with low-GGT (gamma-glutamyl transpeptidase) cholestasis due to impaired canalicular bile flow. MDR3 disease, caused by biliary injury secondary to low-phospholipid bile, presents later and is associated with high-GGT. Individuals with FIC1 disease tend to have extrahepatic complications and slower progression of disease[4,5]. Individuals with BSEP deficiency are likely to have more severe liver disease and are prone to gallstone disease, portal hypertension, hepatocellular carcinoma, and early liver failure[5,6]. Biliary diversion, or surgical interruption of the enterohepatic circulation (sEHC), may improve pruritus and other features of disease in FIC1 and BSEP deficiency, although efficacy in BSEP deficiency appears to depend on mutation[7–12]. FIC1 and BSEP disease may present with a relapsing and remitting course characterized by cholestasis and pruritus, commonly referred to as benign recurrent intrahepatic cholestasis (BRIC). MDR3, in milder forms, can present with a wide range of hepatopathies; nonspecific biliary injury and intrahepatic lithiasis, portal hypertension, and cirrhosis may be present in adolescents or young adults with more severe disease[13].

Recognizing relationships between genotype, phenotype, and clinical course is necessary to improve treatment for PFIC[14]. We sought to further characterize clinical features of genetically-defined FIC1, BSEP, or MDR3 disease from the Childhood Liver Disease Research Network (ChiLDReN) Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis (LOGIC). Specifically, we sought to examine aspects of these diseases, including their clinical presentation and progression, genotype/phenotype correlations, frequency and success rates of sEHC, and frequency of LT.

METHODS

Participants and Study Design

Participants were enrolled into the LOGIC protocol (NCT00571272), a component of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-funded multicenter ChiLDReN research consortium, dedicated to the study of pediatric cholestatic liver diseases, with clinical sites in the United States and Canada. LOGIC is a longitudinal study designed to investigate presenting features, natural history, and genetic correlates of disorders, including Alagille syndrome, alpha-1 antitrypsin deficiency, bile acid synthetic defects, and PFIC in participants <25 years of age at enrollment (inclusion criteria for PFIC/BRIC shown in Supplemental Digital Content [SDC] Table 1). The LOGIC protocol was approved by the Institutional Review Boards at each participating center and conformed to ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from parents or guardians of participants, or from participants themselves (if age 18 years or older). Assent was obtained from participants 7 years of age and older, per local guidelines.

Participants were enrolled in the LOGIC protocol between Nov 2007 and Dec 2013. Inclusion criteria for the PFIC/BRIC diagnostic subgroup in LOGIC are listed in SDC Table 1. These entry criteria were developed to capture a broad phenotypic range from BRIC (mild) to PFIC (persistent and progressive). Some participants met both clinical and genetic criteria for PFIC at LOGIC enrollment, while others met only clinical criteria (SDC Table 1). For those meeting clinical criteria alone, genotyping was performed by the ChiLDReN Genetics core. We performed a cross-sectional multi-center analysis at enrollment that

included relevant medical history, physical examination, laboratory results, and results from imaging studies.

Genetic Analysis

Leukocytes for DNA extraction were obtained either at the baseline visit or at a yearly follow-up visit, and de-identified and coded. Sequencing took place over several years, during which time methodology changed. Most sequencing was performed using Sanger sequencing and ABI technology. Later work was performed using next-generation sequencing (NGS). Illumina TSCA (TruSeq Custom Amplicon) capture was used, with sequencing being performed on an Illumina MiSeq.

NGS data analysis was performed using CLCBio (Qiagen) software, supplemented by Alamut (Interactive Biosoftware) and a locally developed tool for the detection of exonic deletions. For all sequencing methods, the significance of variants was assessed using Combined Annotation Dependent Depletion (CADD) version 1.3[15], as well as minor allele frequencies from the Genome Aggregation Database (gnomAD) and reference to published literature[16].

Participants included in this analysis were selected from the LOGIC PFIC/BRIC cohort based on mutation analysis and were restricted to those with biallelic mutations in *ATP8B1* (FIC1 deficiency) or *ABCB11* (BSEP deficiency), or those with at least one mutated allele in *ABCB4* (MDR3 deficiency). Because monoallelic mutations in *ATP8B1* and *ABCB11* have been reported primarily in asymptomatic individuals (“carriers”), or those diagnosed with intrahepatic cholestasis of pregnancy but previously asymptomatic, individuals with monoallelic mutations in *ATP8B1* or *ABCB11* were excluded from this analysis[17,18]. Cholestatic and/or fibrosing chronic liver disease have been well-described in individuals with monoallelic mutations in *ABCB4* and, as such, participants with either monoallelic or biallelic mutations in *ABCB4* were included. Participants with other genetic forms of cholestasis (including *TJP2* deficiency) were excluded[19,20].

Prior publications (SDC Tables 2–4) focus on evidence from patients, rather than from *in vitro* studies. Publications were identified using the Human Genome Mutation Database (HGMD), ClinVar, and PubMed, facilitated by familiarity with the literature[21,22]. For *ATP8B1* and *ABCB11*, only references reporting mutations in individuals with disease along the continuum from PFIC to BRIC are included. For *ABCB4*, a more liberal approach was taken; references include individuals with a greater variety of phenotypes, cited as evidence of reduced function. Mutations reported as biallelic were confirmed or presumed biallelic; we were unable to verify phase in some participants.

Data and Statistical Analysis

Descriptive statistics performed included means with standard deviations or medians with first and third quartiles for continuous variables, and frequencies and percentages for categorical variables. Comparisons of continuous variables among PFIC subgroups were tested with Kruskal-Wallis tests; comparisons of categorical variables were tested with chi-square tests. Scatterplots displaying laboratory values, anthropometric indices, and years between symptom onset and enrollment based on age were generated to clearly characterize

a heterogeneous cohort containing participants presenting at broadly ranging ages and disease stages. Clinical response to sEHC was assessed, limiting evaluation of sEHC to participants that had sEHC at least 1 year prior to enrollment in the study with their native liver. Clinical parameters used to assess response included total bilirubin, pruritus, and platelet count. Participants were considered to have a successful response to sEHC if the following criteria were met: total bilirubin <=1.2, no or mild pruritus, and platelet count >=150 (or, in those without platelet count reported, spleen size <=2 cm below costal margin)[23]. Statistical analyses were carried out using SAS version 9.4 (SAS Institute; Cary, NC).

RESULTS

Participant Characteristics

Among 209 participants, 171 had genotyping for *ABCB4*, *ABCB11*, and/or *ATP8B1*, with 112 having at least 1 mutation (Figure 1). Fourteen were excluded for monogenic. mutations in ATP8B1 (N=8) or ABCB11 (N=6), leaving 98 participants for analysis with pathogenic variants: 26 FIC1 (*ATP8B1*), 53 BSEP (*ABCB11*), and 19 MDR3 (4 monoallelic, 15 biallelic *ABCB4*). Seventy-six participants were enrolled with their native liver, and 22 enrolled post-liver transplant (post-LT); 35 had history of surgical interruption of the enterohepatic circulation (sEHC), 25 still with native liver and 10 who subsequently underwent LT (2/2 transplanted FIC1 and 8/19 transplanted BSEP).

Parent-reported age at symptom onset was between 0–36 months for all FIC1 and BSEP disease participants (SDC Table 5, SDC Figure 1). Symptom onset was later and more variable for MDR3. Parental report of symptoms and complications at or before enrollment (Figure 2) included pruritus and jaundice in the majority of participants in all three disease subtypes. Pruritus was reported more commonly among FIC1 and BSEP than in MDR3. Other parent-reported symptoms and complications included diarrhea, failure to thrive, bleeding (gastrointestinal [GI] or other)/bruising, bone fracture, and rickets, each of which were more common in FIC1 and BSEP than in MDR3. Ascites at enrollment, as defined by the use of diuretics, was not reported in FIC1 or BSEP, but was present in three MDR3 participants (16%). A history of gallstones was reported for several BSEP and MDR3 participants, but only one FIC1 participant. Hepatocellular carcinoma was reported in two pre-transplant BSEP participants at baseline of just over 1 year of age and suspected in a third post-transplant BSEP participant who was also post-drainage procedure (10-year interval between disease presentation and enrollment). History of pancreatitis was not reported in any participants.

Laboratory and Clinical Data at Enrollment

Anthropometrics obtained at the baseline visit in participants with native liver included weight-for-age, length-for-age, and body mass index (BMI)-for-age z-scores (latter only in participants ≥2 years of age; SDC Figure 2A). Forty-eight percent (11/23 with weight available) of FIC1 were underweight (weight z-score ≤−2; 5 with z-score <−3). Underweight was uncommon in BSEP (2/32 with weight available; 6%) and MDR3 (no underweight participants). Stunting (length-for-age z ≤−2) was common in FIC1 (10/22; 45%) and

present in BSEP (7/31; 23%), but only observed once (6%) in MDR3. Baseline laboratory indices in native liver participants are shown in Figure 3 and SDC Figures 3A–3B. The highest total bilirubin levels were present in FIC1 and BSEP participants, primarily during infancy. Mean ALT was highest in BSEP deficiency (163 U/L), primarily in participants <24 months of age. GGT levels were low (<100 U/L), as expected, in FIC1 and BSEP participants, and highly variable for MDR3 participants. Six of eight MDR3 participants who had GGT levels of <100 U/L at baseline were receiving ursodeoxycholic acid. Total serum bile acid levels varied across age groups and diagnostic subgroups. Platelet counts varied substantially and were highest in infants. Thrombocytopenia was uncommon in FIC1 (2/20 with platelet count available; 10%) and BSEP (5/29; 17%), but frequent in MDR3 (10/17; 59%). All MDR3 participants with thrombocytopenia were >5 years of age.

Gene Mutations

FIC1 (*ATP8B1*) Deficiency—Of the 26 participants identified with biallelic *ATP8B1* mutations (SDC Table 2), 18 different mutations were identified, seven (39%) of which were not previously reported. Ten participants were homozygous for p.G308V (participants 1–10), the mutation first identified in Amish children with PFIC[1]. Five participants carry one copy of the p.I661T mutation (participants 11–15) frequently seen in people of European ancestry with BRIC or PFIC[1,4,24–29]. One of these participants (Patient 11) was heterozygous for p.G308V and p.I661T, and carried a 3rd heterozygous variant, p.S407N, that is not present in gnomAD, which is predicted to be functionally deleterious. Parental genotyping indicates that p.I661T and p.S407N were inherited from the same parent. It is possible that all three of these variants may contribute to decreased function of FIC1 in this patient. An additional mutation, p.D554N, was present in three participants, in this case, in homozygous form (participants 16–18). This mutation has previously been reported in PFIC patients of Inuit ancestry[26,30].

BSEP Deficiency (*ABCB11*)—In the 53 participants with biallelic *ABCB11* mutations (SDC Table 3), 59 distinct mutations were detected, of which 21 (36%) were not previously reported. The mutation most often detected was p.E297G, frequently reported in PFIC participants with European ancestry, and reported in association with relatively good outcomes following sEHC[7,8]. This mutation was present in eight participants (15%) in this study (participants 1–8). Five other mutations, all previously reported, were detected in 3 or more participants in this study (participants 4 and 9–25. Another mutation common in people with European (especially Polish) ancestry, p.D482G, was detected in only two participants in this study (participants 26 and 27). This mutation may confer partial loss of function, yielding a relatively mild phenotype[5,7,8].

MDR3 Deficiency (*ABCB4*)—Of the 19 participants with biallelic or monoallelic *ABCB4* mutations (SDC Table 4), 25 distinct mutations were detected, of which 15 (60%) were not previously reported. Five participants (participants 1–5) were homozygous for a mutation, 10 (participants 6–15) were compound heterozygous for two mutations; in four participants, mutation on only one allele was detected (participants 16–19). Only two mutations, both previously reported, occur in more than one participant; p.R176W and p.G773V each occur in two compound heterozygous participants (participants 7 and 8, 10

and 14, respectively). Participant 14 is compound heterozygous for p.G773V and p.A934T. The latter variant has been previously reported in *ABCB4*-related disease, but may confer partial loss of function, and occurs more frequently than typically expected for a fully penetrant PFIC disease mutation, in individuals of African ancestry (allele frequency 0.014). Participant 10, with relatively late onset of symptoms at 11 years of age, is compound heterozygous for p.A254T, previously reported in a child with idiopathic gallstones, and p.G773V[31]. Three of the four heterozygous MDR3-deficient participants had previously unreported mutations, including p.1263V, p.T1077M, p.G1091V.

Genotype-Phenotype Relationships

To examine relationships between type of genetic mutation and disease severity by transplant and outcomes after sEHC, participants were grouped according to the gene affected and subtype of gene mutation (SDC Table 6). FIC1 was not subcategorized. BSEP was subcategorized into severe (Group 1, two truncating mutations), mild (Group 2, at least one allele E297G or D482G), and other (Group 3, not severe or mild). MDR3 was subcategorized as mono- or biallelic.

A larger proportion of participants with BSEP disease were enrolled post-transplant than either FIC1 or MDR3 (BSEP 19/53, FIC1 2/26, MDR3 1/19). The number of transplanted participants were similar between severe and mild groups. Indications for transplant are not known, as they are not captured in the LOGIC database. Half (four out of eight) participants with severe BSEP mutations had undergone LT. Five of the 10 with “mild” BSEP mutations had also undergone LT.

History of sEHC at least 1 year before enrollment (without subsequent transplant) was examined in FIC1 and BSEP participants (Table 1; SDC Table 6). Four of 10 with FIC1 had successful sEHC. No severe BSEP had sEHC. One mild BSEP had sEHC. Three of eight other BSEP had successful sEHC. Two native liver participants with MDR3 (one monoallelic, one biallelic) had unsuccessful sEHC; their diagnosis had been indeterminate at the time of sEHC.

DISCUSSION

Herein, we describe disease features at enrollment, and mutation data, in a large cohort of infants and children diagnosed with FIC1, BSEP, or MDR3 deficiency. Onset of symptoms, occurring primarily during infancy for FIC1 and BSEP disease, and variable but older onset for MDR3, is similar to that in previous reports[5,13,29,32]. In this cohort, FIC1 and BSEP disease were typically characterized by presence of signs/symptoms likely to be attributed to cholestasis, including pruritus, jaundice, failure to thrive, bone fracture, and rickets. In comparison, participants with MDR3 deficiency more often experienced signs of progressive hepatic fibrosis and portal hypertension, including ascites, thrombocytopenia, and GI hemorrhage. Growth failure was a prominent aspect of disease in FIC1 deficiency. Elevated total bilirubin and associated jaundice appears more common in younger children with FIC1 or BSEP disease. Elevated ALT was a feature of very early childhood in BSEP deficiency.

Severity of disease can be inferred from features of portal hypertension, along with response to sEHC and need for LT. A sense of progression of disease with evolving reduction in platelet count, presumably secondary to portal hypertension in older participants, was identified. Response to sEHC in participants with FIC1 or BSEP disease was modest in our cohort, with about one-third (40% FIC1 and 33% BSEP) meeting our multi-faceted criteria for successful surgery (Methods). Participants with severe BSEP mutations did not typically undergo sEHC, which may reflect the presumption that this approach will not work in BSEP disease with a more severe disease phenotype. We cannot draw conclusions about success of sEHC in BSEP disease with E297G or D482G, mutations previously associated with higher rates of good response to sEHC, as only one participant in our cohort, with p.E297G (and none with p.D482G) underwent sEHC, and this was not successful by our criteria[7,8]. A history of LT was most common in children with BSEP deficiency, consistent with published data[5,13]. One surmises that the severity of liver disease is worse for BSEP deficiency, although absence of information regarding the indication for transplant should be taken into consideration vis-a-vis this interpretation of the data.

We sought to determine genotype-phenotype relationships in these forms of monogenic cholestasis. Despite participation of 15 centers over a 6-year period, the number enrolled with genetically-confirmed disease reflects the rarity of these conditions in North America and the necessity to utilize a prospective multi-center approach to advance understanding of pathogenesis, clinical features, and outcomes. The Amish population served by some of the ChiLDReN sites increased the FIC1 participant numbers, leading to 10 of 26 being homozygous for the classical G308V mutation. Overall, we observed a total of 102 mutations in our cohort, including a significant number of previously-unpublished mutations. Wide variation in mutations complicates determination of genotype-phenotype relationships. Variations in clinical practice, especially relating to sEHC, as well as LT (its availability and indications for its application), both of which interrupt the natural history of the disease, compound the challenge of interpreting genotype-phenotype correlation. In this cohort, 50% of individuals with “milder” *ABCB11* (BSEP) mutations underwent LT. It is not clear whether this reflects true disease course or variability in clinical practice. Also, nearly all of these participants were heterozygous for a “mild” mutation in combination with another, likely more severe mutation; only two of 10 participants carried D482G, likely the milder of these two mutations. As information about risk of malignancy and post-transplant recurrent disease evolved, approaches to clinical decision-making for children with BSEP disease may have changed over the course of this study[6,33].

Analysis was conducted in the context of a multi-center, prospective clinical study with research coordinator-driven data collection, a significant strength of this study. As a result of the relatively large cohort of participants who were enrolled at numerous clinical centers located across North America, this cohort provides important insight regarding the relative frequency of each of the three disorders; in particular, the relatively higher frequency of BSEP deficiency compared with FIC1 and MDR3 deficiency. Interpretation of data in this cohort is complex, in that three distinct genetic disorders (albeit with overlapping phenotypes) are included, and the presentation and course of disease within each diagnostic subgroup is known to be highly variable[26,29,32,34,35]. Additionally, participants were enrolled at a wide variety of ages (from infancy to 18 years of age) and disease stages.

As such, the data presented consist of a single “snap-shot” in time of a heterogeneous group of participants. Data is presented relative to age at enrollment, which gives a sense of the natural history of the disease, although it is limited because this is not a longitudinal study.

In conclusion, we have reported prospective baseline data in one of the largest cohorts of children with diagnoses of FIC1, BSEP, or MDR3 deficiency. We found early disease onset and pruritus predominant in FIC1 and BSEP disease, thrombocytopenia predominant in MDR3, and failure to thrive most commonly in FIC1. LT was most common in BSEP, particularly in those with severe (biallelic truncating) mutation type. sEHC may be of modest benefit or only successful in select participants. Collaborative multi-disciplinary care of children with suspected cholestatic liver disease, beginning with prompt referral to a GI subspecialist by the pediatrician and engagement of the clinical genetics team, will improve care of these patients. Genotype-phenotype relationships revealed through ongoing research will enable thorough screening for disease complications in at-risk individuals, and physicians will be better able to educate families about disease prognosis. Multi-center international study of the variety of mutations discovered in *ATP8B1*, *ABCB11*, and *ABCB4* will be necessary to increase our ability to personalize medicine in these important pediatric cholestatic liver diseases. As such, we will be able to more accurately anticipate disease complications and provide prognostic information for families, and to better inform decision-making with regard to transplant and non-transplant operations for these disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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WHAT IS KNOWN

- FIC1, BSEP, and MDR3 deficiency typically present in infancy or childhood.
- Non-transplant surgery (sEHC) is performed in some patients to alleviate pruritus and/or slow disease course.
- A variety of causative gene mutations are known; novel mutations continue to be discovered.

WHAT IS NEW

- Cross-sectional genetic and clinical data, including 43 novel mutations, from a multi-center North American research consortium are presented.
- Using multi-factorial criteria to define good response to sEHC, this procedure had modest success rates, overall, in patients with FIC1 or BSEP deficiency in this cohort.
- Genotype-phenotype correlations are difficult to determine and will likely require international collaborative efforts.

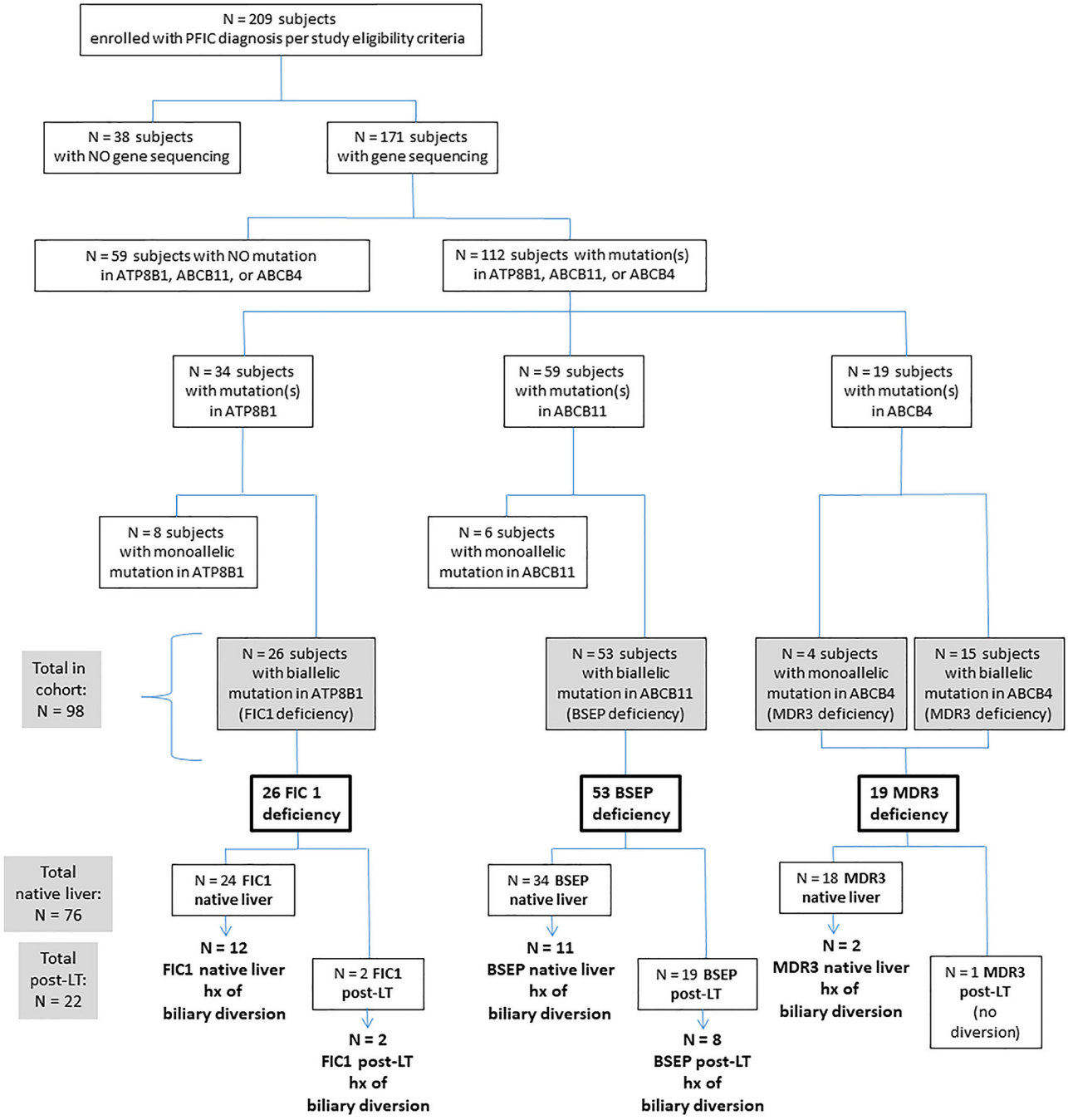

Figure 1.

Participants included in study cohort. Flow diagram shows composition of final cohort (N=98 as indicated in shaded box on left) selected, based on gene sequencing, from 209 children with a clinical diagnosis of progressive intrahepatic cholestasis. Abbreviations: hx, history; N, number of participants; post-LT, post-liver transplant; sEHC, surgical interruption of the enterohepatic circulation.

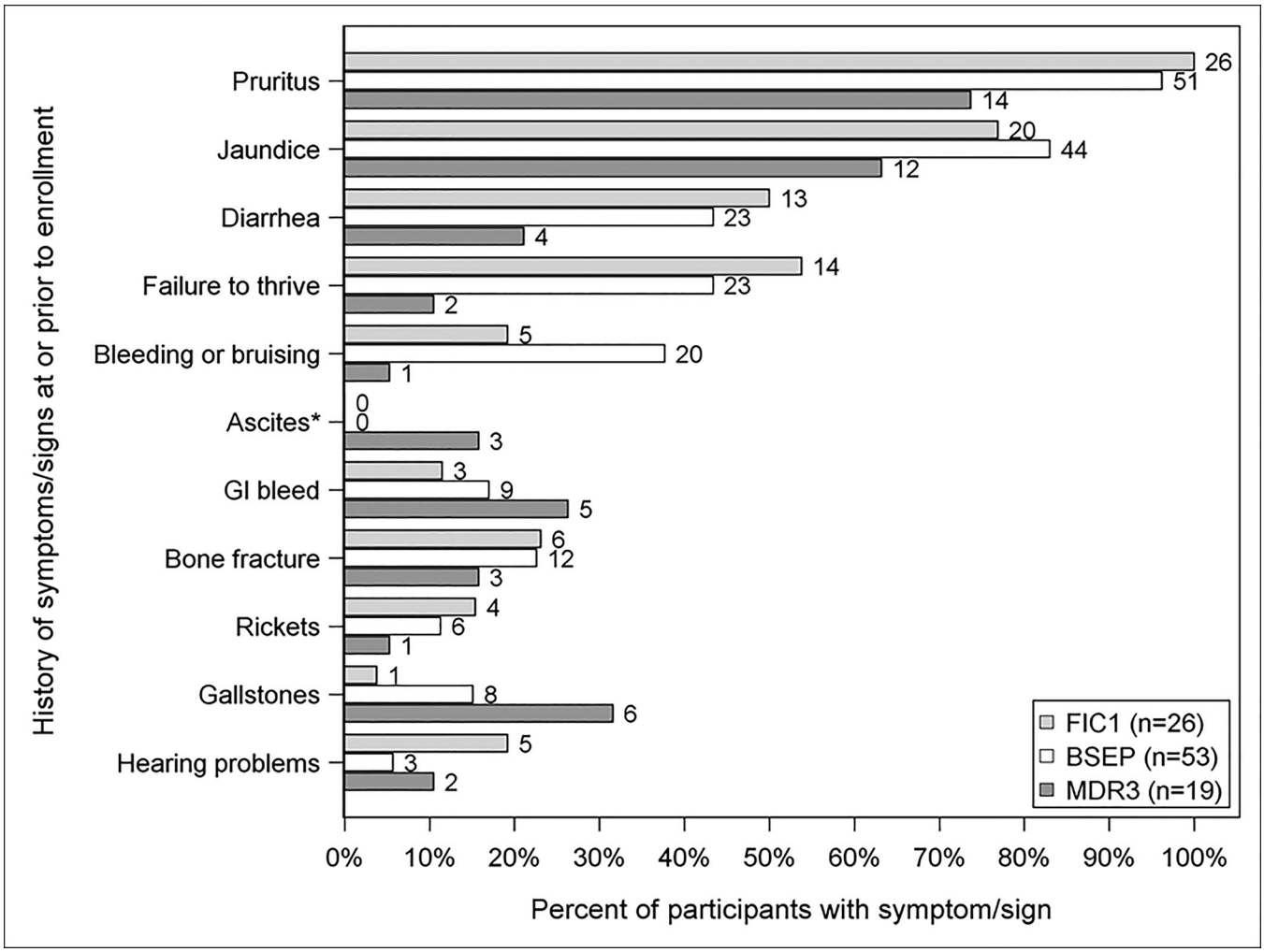

Figure 2.

Symptoms reported at or before enrollment. Bar graph shows number (at end of each bar) and percentage (x-axis) of subjects with parent-reported history of each of the symptoms listed along the y-axis. Key indicates shading of bars to distinguish subjects with each genetic diagnosis. Abbreviations: BSEP, bile salt export pump; FIC1, familial intrahepatic cholestatis; GI, gastrointestinal; MDR3, multidrug resistance.

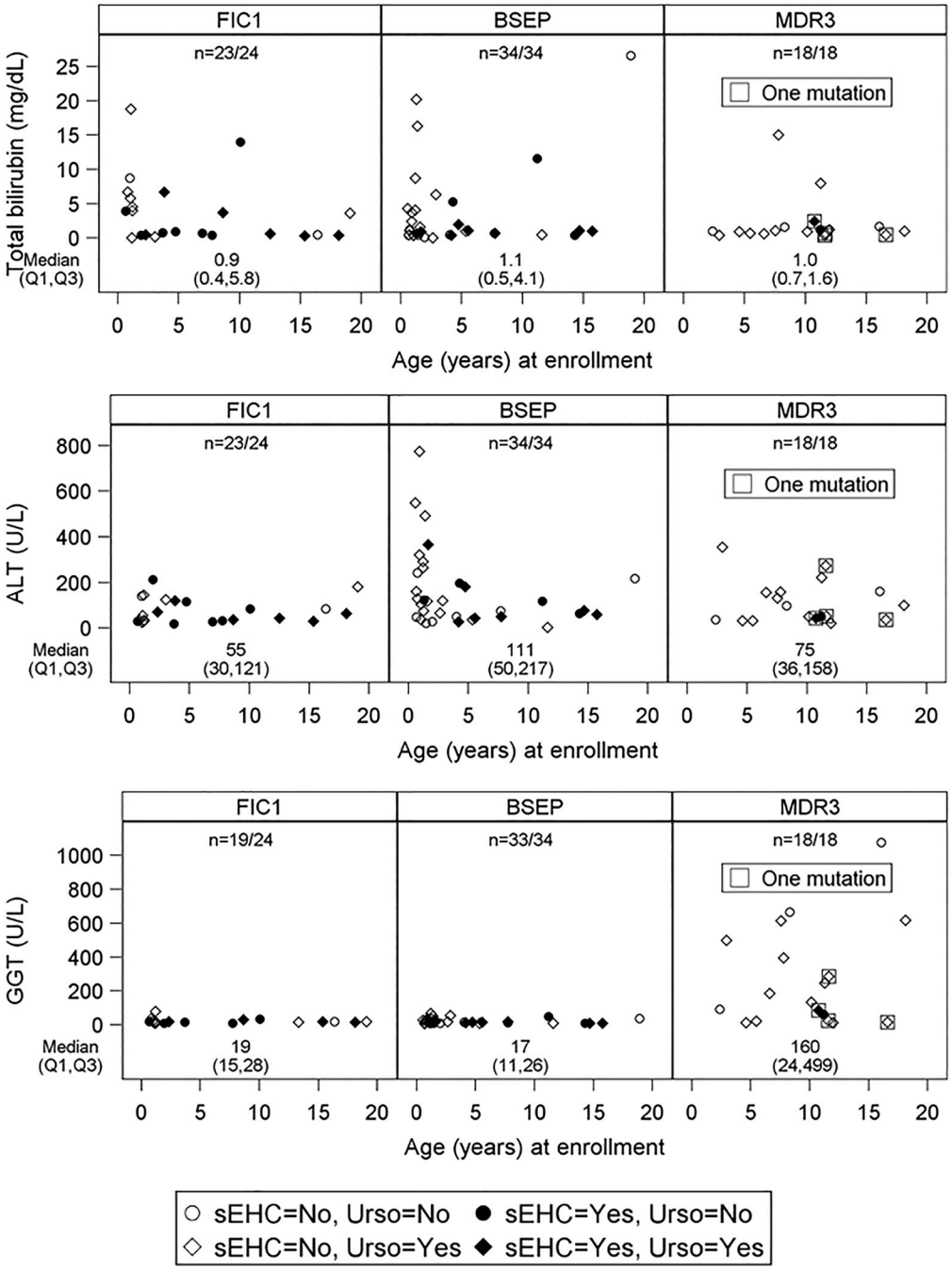

Figure 3.

Liver biochemistries at enrollment for participants with native liver. Scatterplots show laboratory test (y-axis), age in years (x-axis), and total bilirubin, ALT, and GGT divided according to FIC1, BSEP, or MDR3 disease. Horizontal line extending across the GGT chart represents the upper limit of normal GGT of 100 U/L. Each marker represents value at enrollment for one participant. Key indicates participants with ("sEHC=Yes") or without ("sEHC=No") history of sEHC, and those taking ("Urso=No") or not taking ("Urso=No") ursodeoxycholic acid. For MDR3 disease, a box around marker indicates participants with

one mutation; all others (in all three disease groups) have biallelic mutations. Abbreviations: ALT, alanine aminotransferase; BSEP, bile salt export pump; FIC1, familial intrahepatic cholestatis; GGT, gamma-glutamyl transpeptidase; MDR3, multidrug resistance; sEHC, surgical interruption of the enterohepatic circulation; Urso, ursodeoxycholic acid.

Table 1.

Native liver participants with history of sEHC at least 1 year prior to enrollment. Number of participants in each disease group enrolled pre-transplant, as well as such participants specifically with history of *sEHC 1 year or more before enrollment*, are indicated. Participants are stratified based on total bilirubin, presence/severity of pruritus, and platelet count, and as having "successful drain" per the definition shown under table.

n (%) or mean (standard deviation)	FIC1	BSEP	Total
Participants enrolled pre-transplant	24	34	58
Participants with drainage procedure performed at least 1 year prior to baseline	10 (42%)	9 (26%)	19 (33%)
Time (years) between drainage procedure and baseline	7.2 (4.2)	7.2 (4.6)	7.2 (4.3)
Total bilirubin (mg/dL)			
≤1	7 (70%)	4 (44%)	11 (58%)
>1	3 (30%)	5 (56%)	8 (42%)
Pruritus			
None or mild	5 (50%)	5 (56%)	10 (53%)
Active scratching or cutaneous mutilation	5 (50%)	4 (44%)	9 (47%)
Platelet count (10^3/mm^3)			
<150	2 (20%)	3 (33%)	5 (26%)
≥150	6 (60%)	4 (44%)	10 (53%)
Missing	2 (20%)	2 (22%)	4 (21%)
Successful sEHC *			
No	6 (60%)	6 (67%)	12 (63%)
Yes	4 (40%)	3 (33%)	7 (37%)

* Successful sEHC defined as total bilirubin ≤1, no or mild pruritus, and platelet count ≥150, or spleen size ≤2cm below costal margin when platelet count is missing.

Abbreviations: BSEP, bile salt export pump; FIC1, familial intrahepatic cholestasis; sEHC, surgical interruption of enterohepatic circulation.